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Functional Implications of DNA Methylation in Adipose Biology

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The twin epidemics of obesity and type 2 diabetes (T2D) are a serious health, social, and economic issue. The dysregulation of adipose tissue biology is central to the development of these two metabolic disorders, as adipose tissue plays a pivotal role in regulating whole-body metabolism and energy homeostasis (1). Accumulating evidence indicates that multiple aspects of adipose biology are regulated, in part, by epigenetic mechanisms. The precise and comprehensive understanding of the epigenetic control of adipose tissue biology is crucial to identifying novel therapeutic interventions that target epigenetic issues. Here, we review the recent findings on DNA methylation events and machinery in regulating the developmental processes and metabolic function of adipocytes. We highlight the following points: *1*) DNA methylation is a key epigenetic regulator of adipose development and gene regulation, *2*) emerging evidence suggests that DNA methylation is involved in the transgenerational passage of obesity and other metabolic disorders, *3*) DNA methylation is involved in regulating the altered transcriptional landscape of dysfunctional adipose tissue, *4*) genome-wide studies reveal specific DNA methylation events that associate with obesity and T2D, and *5*) the enzymatic effectors of DNA methylation have physiological functions in adipose development and metabolic function.

EPIGENETIC CHANGES ARE LINKED TO OBESITY AND TYPE 2 DIABETES

Obesity and type 2 diabetes (T2D) are highly complex human diseases, and genetics plays an important role in the etiology of both. With the advent of next-generation sequencing, several common single nucleotide polymorphisms (SNPs) have been discovered in association with disease susceptibility. However, the vast majority of these variants have not been tested for causality, and even if proven causal, they cannot fully explain many clinical features such as high heritability, high discordance in adult monozygotic twins, and the close relationship with environmental factors (2–5). Therefore, it has long been speculated that nongenetic variation, such as epigenetic alterations, plays a role in pathogenesis. This notion has been borne out by a recent epigenome-wide association study that linked alterations in DNA methylation to whole-body insulin sensitivity (6).

DNA methylation is a reversible epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of a cytosine residue by DNA methyltransferases (DNMTs), usually in the context of a cytosine-guanine dinucleotide (CpG) doublet. Though methylated DNA has long been thought to be a static mark, recent studies indicate that methylated DNA undergoes dynamic and reversible remodeling through DNA demethylases, namely, the ten-eleven translocation (TET) proteins. Mounting evidence supports that DNA methylation is involved in various forms of metabolic perturbation, from the abnormal development of adipose tissue to the dysfunction of adult adipocytes. Here, we will discuss the DNA methylation events that impact metabolism and the functional roles of DNMTs and TET proteins in adipose biology, with an emphasis on those that may be associated with obesity and T2D.

DNA METHYLATION AND ITS MACHINERY

DNA methylation is a process by which methyl groups are added to the DNA molecule, especially at the 5 carbon of the cytosine ring, which forms 5-methylcytosine (5mC) (7). In mammals, 5mC is mostly found in the context of paired symmetrical methylation of a CpG site, a site in which a cytosine is located next to a guanidine (7). However, non-CpG methylation is also detected in human

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and other species (8–10). In the bulk of genomic DNA, most CpGs are methylated, whereas those located in a CpG island (where CpG sites cluster to form repetitive sequences) remain largely unmethylated (7).

DNA methylation is mediated by DNMTs. In mammals, five family members of the DNMT proteins have been characterized—*Dnmt1*, *-2*, *-3a*, *-3b,* and *-3L*—yet only the first three possess DNMT activity (11). DNMT1 is the maintenance Dnmt for replication, whereas DNMT3a and -3b are referred as de novo DNMTs, as they can establish a new DNA methylation pattern (11). The DNMT3-like protein *Dnmt3L* is homologous to the other *Dnmt3s* but lacks catalytic activity, and *Dnmt2* has sequence homology to all Dnmts but methylates cytoplasmic tRNA instead of DNA (11).

DNA methylation has long been thought to be a static epigenetic mark, but emerging evidence suggests that it undergoes dynamic and reversible remodeling in somatic cells during developmental and pathogenic processes (12,13), making its machinery and effects attractive drug targets. For example, DNA methylation can be erased by either passive or active mechanisms or a combination of both (14). Passive demethylation is often due to the loss of 5mC during successive rounds of replication in the absence of methylation maintenance machinery such as DNMT1. By contrast, active demethylation is mediated by a set of enzymes; TET proteins (TET1, -2, and -3) oxidize 5mC to hydroxymethylcytosine (5hmC), which is then converted to unmethylated cytosine (5C) through base excision repair and thymidine DNA glycosylase (15).

The biological importance of DNA methylation as a major type of epigenetic modification in regulating gene expression has been well established. In general, reduced DNA methylation in the promoter or other gene regulatory regions is associated with increased DNA binding of transcription factors and chromatin proteins, thus allowing gene transcription to occur (16). By contrast, increased DNA methylation at the regulatory regions is often associated with gene repression (17).

DNA METHYLATION IN ADIPOGENESIS

DNA methylation plays an important role in a broad scope of developmental processes including adipogenesis (18–21). Inhibiting DNMT in multipotent C3H10T1/2 cells and 3T3-L1 preadipocytes stimulates spontaneous differentiation and enhances differentiation in response to adipogenic inducers (22,23). However, genetic studies have conflicting results with regard to the exact role of DNMTs in adipogenesis. DNMT1 is crucial for maintaining DNA methylation and repressive histone H3K9 methylation patterns prior to differentiation, suggesting it represses 3T3-L1 adipogenesis (24). However, knocking down Dnmt1 and -3a impairs 3T3-L1 adipogenesis (25). This discrepancy might be due to the experimental conditions and tissue culture variables between the two laboratory environments. In another controversy, it was found that, in late-stage differentiation, DNMT inhibition promoted lipid accumulation by enhancing lipogenesis by upregulating the lipogenic transcription factor *Srebp1c* (25). By contrast, other groups reported that DNMT inhibition reduced adipogenic capacity in 3T3-L1 and ST2 mesenchymal precursor cell lines by upregulating canonical Wnt signaling (21,26).

Fortunately, there is more consensus in the field about the DNA methylation profile of key adipocyte genes during differentiation (Fig. 1). PPARγ is expressed mainly in adipose tissue, where it regulates fatty acid storage and glucose metabolism, and C/EBP is involved in adipogenesis. The promoters of both genes are gradually demethylated during 3T3-L1 adipogenesis, correlating with increased expression of the genes (27,28). This coincides with the loss of repressive histone marks (H3K9me3) and the gain of active marks (e.g., H3K27ac and H3K4me3) (28), although the regulation of this timing is not clearly

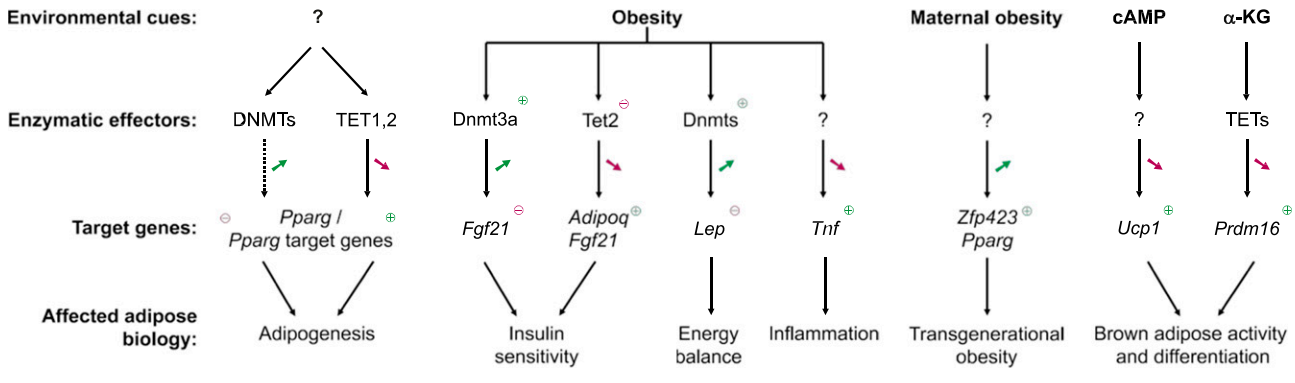

Figure 1—Summary of the relationship between environmental factors and DNA (de)methylation machinery in the regulation of adipose biology. Various perturbations from environmental cues affect the expression or activity of DNMTs and TETs, which alters the DNA methylation profile of specific target genes with concordant changes in gene expression and phenotypic changes in adipose biology. The dotted line is used to depict relationships with weaker evidence. Small arrow inserts indicate the direction of change in DNA methylation at affected genes. Plus and minus signs indicate the direction of gene expression change.

understood. In addition, dexamethasone treatment causes *Cebpa* demethylation in C3H10T1/2 cells, with a concordant release of DNMT3a and -3b from the promoter (28). It is also noteworthy that the gene bodies of both *Pparg* and *Cebpa* are highly hypermethylated in embryonic stem cells to restrict lineage commitment to adipogenesis (29). Similarly, reduced DNA methylation is observed during adipogenesis at the promoters of other adipocyte genes (e.g., *Lep* [30], *Slc2a4* [GLUT4] [30], and *Peln* [27]). Intriguingly, recent global profiling studies have demonstrated that 5hmC (a cytosine that is an intermediate product of DNA demethylation) colocalizes with PPARγ at enhancers in 3T3-L1 adipocytes (31), and PPARγ-positive nuclei sorted from visceral adipose tissue from healthy humans are strongly coenriched with 5hmC (32). Given that the TET proteins, especially TET1 and TET2, are necessary for adipose conversion (33) and that PPARγ physically interacts with them (27,34) indicate that PPARγ is influencing the methylation pattern.

DNA methylation has also been implicated in the transgenerational regulation of adipose development. Maternal obesity predisposes offspring to obesity and T2D, yet the mechanisms remain unknown. A rodent study demonstrated that maternal obesity increases the expression of Zfp423 (the key transcription factor committing cells to the adipocyte lineage [35] and maintaining white adipocyte identity [36]), which results from hypomethylation at the promoter region of Zfp423, which has exceptionally high density of CpGs in the promoter (37). Increased Zfp423 expression implies increased adipose expansion but fewer beige/brown adipocytes, which can contribute to increased adiposity during fetal development and metabolic dysfunction later in life. Another mechanism for transgenerational regulation could be PPARγ, which has reduced expression and function in obesity and adipose metabolic dysfunction. Offspring that are born to obese rat mothers have persistently lower PPARγ expression, more epigenetic repression, including DNA hypermethylation, and reduced enrichment of active histone marks at the PPARγ promoter region (38).

DNA (de)methylation governs brown adipocyte–specific gene regulation and development. In contrast to white adipocytes, which store excess energy in the form of triglycerides, brown and beige adipocytes dissipate energy in the form of heat. While white and brown adipose development share a similar genetic cascade, they have distinct transcriptional and epigenetic programs (39). A genome-wide study showed that the overall DNA methylation pattern of white adipocytes is different from that of brown (40). Compared with the white 3T3-L1 cell line, the brown adipocyte cell line HIB-1B has reduced methylation at the CpGs around cAMP response elements, which are important for the sympathetic stimulation of *Ucp1* expression, a marker gene for brown adipocytes that mediates adaptive thermogenesis (41). In 3T3-L1 adipocytes, the corepressor protein RIP140 recruits repressive histone modifiers, such as HDAC1 and -3, and all three DNMTs to the *Ucp1* enhancer and promoter regions for gene repression (42). Consistently, treatment of 3T3-L1 and mouse embryonic fibroblasts with DNMT inhibitor increases *Ucp1* expression. Furthermore, TET-mediated DNA demethylation is required for the gene activation of *Prdm16*, an important transcriptional regulator of brown adipose development (43).

A more recent study reports that DNA methylation is involved in transgenerational regulation of brown and beige adipose activity (44). Interestingly, paternal cold exposure before mating results in improved systemic metabolism and protection from diet-induced obesity of the male offspring. Such transgenerational impact of cold exposure through male lineage was associated with differential methylation at multipole loci (44). Most prominently, the gene body of *Adrb3*, which encodes a protein mediating β-adrenergic stimulation in brown adipose tissue, was hypomethylated in association with increased gene expression in sperm genomic DNA (44). More studies should be conducted to understand the functional implication of DNA methylation in plasticity between beige and white adipocytes in response to various stimuli. Elucidating the epigenetic mechanisms of brown and beige adipose biology will shed light on effective therapeutic interventions for obesity and obesity-related human diseases.

Notably, a majority of these studies were conducted using tissue culture models. Although the results from in vitro studies provide important insights and are often conserved in vivo, the epigenome can profoundly differ between in vitro and in vivo contexts. Therefore, in vivo studies are required to better understand the physiological role of epigenetics in adipocyte commitment and developmental processes. Also, it will be important to investigate how distinct DNA methylation events interact with other epigenetic and transcriptional regulators to confer genomic target specificity and gene regulation.

DNA METHYLATION IN ADIPOCYTE FUNCTION

In obesity and obesity-related metabolic issues, adipokine regulation is profoundly altered (45), and some of these changes are regulated by DNA methylation (Table 1). Leptin is the key adipokine that mediates adipose tissue–brain communication to maintain energy homeostasis and normal body weight (46). Obesity is typically associated with high leptin levels and results in resistance to leptin (47). So far, the molecular and epigenetic mechanisms underlying that remain largely unknown.

Lep methylation is inversely correlated with adipocyte-specific *Lep* expression. For example, the *LEP* promoter is hypermethylated in the stromal vascular fraction but hypomethylated in the adipocyte fraction of human visceral adipose tissue (48). *Lep* promoter methylation decreases during mouse adipogenesis concurrently with increased *Lep* expression (49). Consistent with this, DNMT inhibition increases *LEP* expression in cell lines such as primary fibroblasts and HeLa cells (48).

Function	Adipogenesis	Obesity	Weight loss	Type 2 diabetes
Bone metabolism	-	*SPP1*	-	-
Energy balance	*LEP*	*LEP*	*LEP*	-
Glucogenesis	-	*PCK1*	-	-
Glucose homeostasis	-	-	-	*FGF21*
Hypoxia response	-	-	-	*HIF3A*
Inflammation	-	*CCL18*	*TNF*	-
Insulin sensitivity	*ADIPOQ*	*ADIPOQ*	-	-
Insulin signaling	-	*AKT2*	-	*IRS1*
Lipid droplet formation	*PLIN*	-	-	-
Obesity related	-	*FTO*	-	-
Potassium channel	-	-	-	*KCNQ1*
Transcription factor	*PPARG*	*TCF7L2, NANOG, OCT4, SOX2*	-	*PPARG*

Table 1—Summary of differential methylated loci in adipocytes in association with obesity and T2D.

Reduced *Lep* methylation could account for obesity-related leptin upregulation (49,50); however, the results have been inconsistent (51,52). Surprisingly, studies suggest that the *Lep* locus is hypermethylated in obesity. A high-fat diet regimen initially reduces *Lep* promoter methylation for up to 8 weeks, yet prolonged high-fat feeding for more than 12 and 14 weeks increases *Lep* methylation, especially in epididymal fat (53,54). This is accompanied by increased occupancy of DNMT1, DNMT3a, and DNMT3b at the *Lep* promoter (54). Similarly, long-term maternal high-fat feeding in rats results in increased birth weight, *Lep* hypermethylation, and increased plasma leptin levels in offspring (51). Diet-induced weight loss in obese female subjects accompanies *LEP* hypomethylation in association with increased *LEP* expression (48). By contrast, bariatric surgery–induced weight loss does not alter *LEP* methylation, though it decreases *LEP* expression (48). These results seem paradoxical and suggest that epigenetic modification occurs as a feedback regulatory mechanism due to increased *LEP* expression but is insufficient to normalize the expression. Further investigations are necessary to draw a general conclusion as to whether and how DNA methylation contributes to leptin gene regulation in various regimens of weight gain and loss.

Adiponectin is a protein hormone mainly produced by adipose tissue and is encoded by the *Adipoq/ADIPOQ* gene in mouse and human (55). It plays an important role in the maintenance of energy homeostasis by regulating glucose and lipid metabolism (56). Reduced circulating adiponectin level is correlated with obesity, insulin resistance, and T2D (55). Consistent with this, the *Adipoq* proximal promoter region is hypermethylated in obese mice (57) due to increased DNMT1 expression and activity. Moreover, systemic administration of DNMT inhibitor rescues adiponectin expression and improves glucose intolerance in high fat–fed wild-type mice (57). Human studies also support that *ADIPOQ* methylation in subcutaneous adipose tissue is positively correlated with BMI, waist girth, and fasting LDL cholesterol in plasma (58). *ADIPOQ* is also hypermethylated in the maternal adipose tissue of obese pregnant women, resulting in significantly lower plasma adiponectin levels (59).

Fibroblast growth factor 21 (FGF21) is well-known as a hepatokine, but it is also expressed in other tissues including fat and muscle (60). FGF21 facilitates glucose uptake in adipocytes (61–63) through unknown mechanisms. Adipocyte expression of *Fgf21* is negatively regulated by Dnmt3a, with concordant changes in DNA methylation in Dnmt3a gain- and loss-of-function models. Consistently, CpGs around *FGF21* are hypermethylated in adipose tissue from T2D patients with a negative correlation with FGF21 expression in adipose tissue (64).

Tumor necrosis factor α (TNFα) was traditionally considered to be secreted chiefly by macrophages, but it is also produced by other cell types including adipocytes (65). It is well established that circulating TNFα levels are positively correlated with insulin resistance in obesity (66). Contrary to what is expected, obese individuals with significant weight loss have decreased methylation at the promoter of *Tnf*. For instance, the obese women who lost more weight in a low-calorie diet intervention displayed lower promoter methylation levels of *Tnf* in adipose tissue (67). Similarly, obese men with significant weight reduction through a balanced-nutrition intervention also showed decreased methylation levels (68). Similar to the case

with *LEP* methylation, hypermethylation at the *Tnf* may occur as an adaptive mechanism to prevent further production of TNFα in obesity.

In addition to these sites, global profiling studies have detected profound changes in DNA methylation at multiple loci in obesity and T2D (Table 1). Studies of monozygotic twins discordant for T2D identified differential methylation at 7,046 genetic loci, including at candidate genes for T2D identified through GWAS such as *PPARG*, *IRS1*, *TCF7L2*, and *KCNQ1* (69). Three independent studies found DNA hypermethylation at the CpGs near *HIF3A* (70–72) in relation to BMI. This gene encodes a protein that is part of the heterodimeric hypoxia-inducible factor (HIF) transcriptional complex, which regulates many adaptive responses to hypoxia. The specific role of *HIF3A* in adipose biology is not well-known, but adipocyte-specific depletion of *Hif1a* in the HIF heterodimer improved insulin sensitivity in the context of a high-fat diet (73).

Additionally, differential methylation has been identified at adipose biology–related genes (e.g., *FTO*, *TCF7L2*, *IRS1*, *CCL18*, and *SPP1*) in obesity and T2D (69). A recent mouse adipocyte methylome study identified a number of differentially methylated regions in diet-induced obesity, some of which negatively correlate with gene expression changes (e.g., *Pck1*, *Tcf7l2*, and *Akt2*) (74). Cross-species analysis identified 170 differentially methylated regions that are conserved in human obesity, and 30 of them (e.g., *Mkl1*, *KCNA3*, and *Etaa1)* overlap with SNPs or nearby proxies that are associated with human T2D genetic risk. The authors integrated the DNA methylome with other chromatin modification maps, transcription localization maps, and disease associations (SNP/expression quantitative trait loci) to reveal DNA methylation events that might be more functionally relevant to disease susceptibility.

Genome-wide studies provide a powerful tool to discover changes in DNA methylation that might be functionally relevant to human obesity and T2D. However, they have a few major limitations. A majority of profiling studies, especially human studies, use an array-based method that covers a fraction of the CpG sites, being biased toward promoters and strongly underrepresenting distal regulatory elements. Base pair–resolution studies will be necessary in the future. Also, it should be noted that there is little overlap among the differentially modified gene loci between studies. Furthermore, it will be of great importance to address the causality of individual methylation events and follow up on the metabolic function of the proteins encoded by affected genes.

DNMT AND TET PROTEINS IN ADIPOSE BIOLOGY

Emerging evidence indicates that DNMTs are directly involved in regulating metabolic function in addition to developmental processes. The basal level of Dnmt1 and -3a is modestly high, while that of -3b is barely detectable in various mouse adipose tissues. Adipose DNMT levels are significantly increased in diet-induced obesity as well as in genetically obese *ob/ob* mice (75). Dnmt3a-overexpressor mice on a high-fat diet have increased expression of inflammatory cytokines such as TNF-α and MCP-1 (75). As discussed earlier, DNMT1-mediated hypermethylation suppresses *Adipoq* expression in obesity (57). Adipose Dnmt3a plays a causal role in the development of insulin resistance in mice, as evidenced by adipose-specific deficiency of Dnmt3a conferring protection from diet-induced metabolic dysregulation independent of body weight or adiposity (64). Future studies should be conducted and followed up to determine whether there is a conserved role for DNMT3A in human insulin resistance.

Consistently, pharmacological DNMT inhibition improves insulin resistance both in vitro and in vivo (57,64), suggesting that DNMT inhibition can be an attractive therapeutic approach for metabolic disorders. Notably, administration of pan-inhibitors of histone deacetylase exerts beneficial metabolic effects in both mice and humans, such as increased energy expenditure, insulin sensitivity, and secretion (76–79). Together, these studies provide proof of principal that targeting epigenetic issues can be considered for therapeutic intervention to approach metabolic disorders.

Emerging evidence suggests that the TET proteins play an important metabolic function in adipocytes. All three TETs are expressed in mouse adipose tissue, but only Tet2 expression is reduced in diet-induced obesity. The expression of TET1 and TET2 is diminished in adipose stem cells from obese subjects, concurrent with a reduction of global 5hmC levels (80). It is noteworthy that global 5hmC levels are downregulated in blood samples from patients with diabetes, and this is dependent on TET2 action (81); this further suggests that altered TET2 action may influence glucose homeostasis. PPARγ and the TET proteins appear to functionally and physically interact (34). During adipogenesis, PPARγ, via the physical interaction with TET1, increases local demethylation around PPARγ-binding sites (27). In mature adipocytes, TET2 facilitates the transcriptional activity of PPARγ and insulin-sensitizing efficacy of PPARγ agonist by sustaining DNA binding of PPARγ at certain target loci (34). It is noteworthy that global 5hmC levels are downregulated in blood samples from patients with diabetes, further demonstrating that TET2 plays a necessary role in maintaining glucose homeostasis as a downstream effector of AMPK, especially in the oncogenic state (81). Together, these studies suggest that TET2 is a critical epigenetic sensor/regulator of glucose in the cell.

CONCLUDING REMARKS

Accumulating evidence suggests that epigenetics, which sits at the interface of genetics and environment, plays a dynamic role in the regulation of metabolic processes. With the reversibility of epigenetic changes, drugs that target these changes hold great promise for the prevention, diagnosis, treatment, and prognosis of metabolic disorders; however, there are still several challenges to overcome (see OUTSTANDING QUESTIONS [below]). First, it is essential to gain a more accurate and comprehensive

understanding of DNA methylation events that are at the core of pathogenesis. Despite a plethora of studies reporting DNA methylation changes in association with disease state, we still lack information about which changes are core to the condition and which events drive the phenotype. Capturing more dynamic changes through base pair–resolution profiling during fine time course studies will be necessary. Second, target specificity needs to be carefully addressed. DNA methylation is involved in a broad spectrum of biological processes in multiple tissues and cell types. Although systemic administration of DNMT inhibitors improves the metabolic profile, it is still under investigation whether they have deleterious effects due to nonspecificity. Target-specific epigenetic editing studies in mouse models have been performed and will be needed to address this question. Third, more thorough biological validation in cells and animals will be critical. Some epigenetic changes may increase disease susceptibility, but some occur as a consequence of the disease phenotype. Thus, functional validation of individual DNA methylation events and machinery should be done to resolve the consequence/causality issue in a definite way. In conclusion, we seek to elucidate the reversible and treatable epigenetic changes that can be used for personalized medicine and targeted therapy for metabolic diseases.

OUTSTANDING QUESTIONS

What triggers the change in DNA (de)methylation and machinery? Epigenetic regulation operates at multiple levels, and little is known about which stimuli (e.g., nutritional signals and molecular and epigenetic regulators) drive the change.

How dynamic is the change in methylation? A vast majority of studies profiled the change between disease and nondisease states, but very few studies have examined reversibility. It is difficult to delineate which changes are initial, and likely contribute to the pathogenesis, and which are consequential. Cataloging the dynamic changes may help narrow down the list to causal and treatable epigenetic changes.

What confers target specificity? DNA methylation machinery does not bind to DNA in a sequence-specific manner. Identifying recruiting factors that target genomic loci will be critical to achieving specificity.

What is the role of DNA methylation in interindividual differences in disease susceptibility and drug efficacy? DNA methylation is highly variable between individuals, even in those with the same genetic content, as evidenced by monozygotic twin studies. Investigating which changes are important to etiology and efficacy may identify new therapeutic approaches.

What is the role of metabolic cofactors in disease-associated DNA methylation? Epigenetics is regulated at multiple levels, and several key metabolites function as cofactors. DNMTs use *S*-adenosyl-L-methionine, generated by the methionine cycle, as the methyl donor, whereas TET enzymes require α-ketoglutarate, a key by-product of the tricarboxylic acid cycle. A change in metabolic state would affect the concentration of these metabolites in cells and modulate the enzymatic activity of DNMTs and TETs. Understanding the regulatory function and mechanism of DNA (de)methylation at the cofactor levels will be crucial for developing a nutritional approach to therapy.

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